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TITLE: Treatment of Traumatic Brain Injury by Localilzed Application of Sub-atmospheric Pressure to the Site of Cortical Impact

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14. ABSTRACT

Each major war tends to have a ósignature injuryô, with traumatic brain injury (TBI) associated with the Iraq war (Operation Iraqi Freedom II and Operation Enduring Freedom) due to the high incidence of personnel injured by IED (improvised explosive devices). The large gyrencephalic brain of swine is similar to humans, thus a swine model of a controlled cortical impact (CCI injury) treated by the controlled application of sub-atmospheric pressure was employed. Work completed in Year 1 showed that 100 mm Hg vacuum application to the site of a focal brain injury decreased the volume of hemorrhage and inured area. Work completed in Year 2 showed that 100 mm Hg vacuum needed to be applied to the site of the focal injury for a minimum of 5 days to prevent late increases in intra cranial pressure and resumption on the injury process. In Year 3, work completed showed that that a 3 hour delay between injury and initiation of vacuum treatment was as efficacious as immediate treatment, and that a 6 hour delay between injury and initiation of vacuum treatment was still efficacious. This allows for a realistic working window for successful application of controlled, ocalized vacuum to the site of a focal TBI.

15. SUBJECT TERMS

Traumatic brain injury, sub-atmospheric pressure treatment, vacuum, mechanical tissue resuscitation

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Introduction

Each major war tends to have a 'signature injury', with traumatic brain injury (TBI) associated with the Iraq war (Operation Iraqi Freedom II and Operation Enduring Freedom) due to the high incidence of personnel injured by IED (improvised explosive devices). Our previous study in a rat model showed that Mechanical Tissue Resuscitation (MTR – the controlled application of vacuum) to the cerebral cortex following a controlled cortical impact (CCI) injury reduces brain edema and the extent of injury, modulates metabolites in injured neuronal tissues, preserves neuronal tissue, and improves functional recovery. The large gyrencephalic brain of swine is similar to humans, thus a swine model of CCI injury and MTR treatment was evaluated for future human clinical applications. Year 3 goals were to determine the length of time between the initial injury event and efficacious application of vacuum.

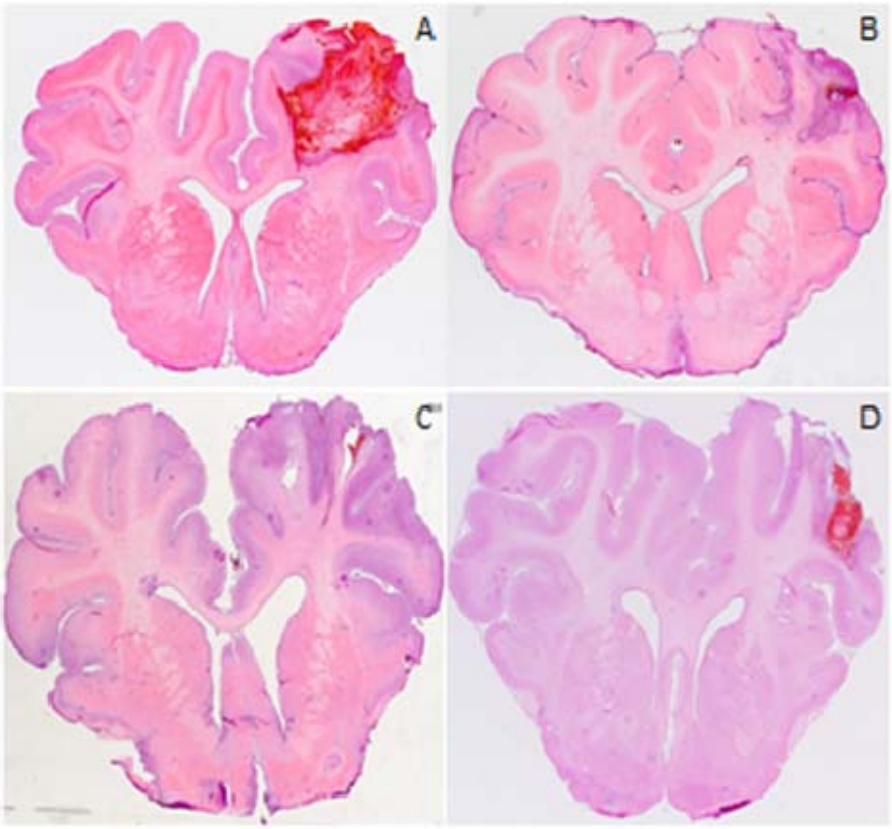
Body

Year 3 goals stated in the Statement of Work included determination of the length of time between creation of a focal TBI and then efficacious application of vacuum application to the site of the focal injury. Injuries were created and animals were treated with 100 mm Hg vacuum immediately (based on Year 1 and 2 studies), or after a 3 or 6 hour delay.

During Year 3 the MRI scanner used for imaging during Years 1 and 2 was removed and replaced with a new, more powerful scanner. This resulted in a hiatus in the project due to the inability to scan.

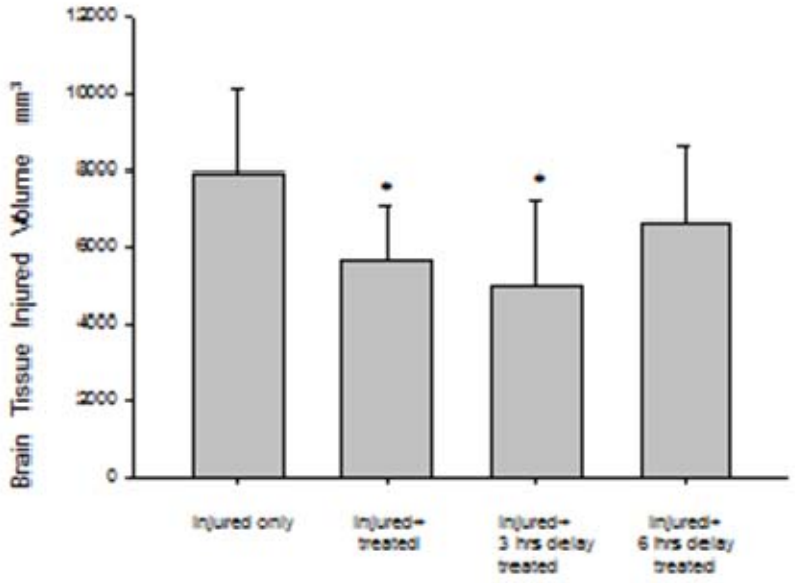
A total of thirty two (32) female domestic swine (22-33 kg) were procured (including those treated immediately (0 hour delay) in previous years). For MTR treatment, a sterile vacuum dressing was placed in the bony defect and 100 mm Hg was applied continuously either immediately post injury, or after either a 3 or 6 hour delay. Vacuum was applied for a total of 5 days. Intracranial pressure was monitored by telemetry up until the time of MR imaging. Five days post surgery, all animals were analyzed by MRI (GE Signa EchoSpeed 1.5-T scanner). Parameters analyzed included: MR sagittal T1 imaging; coronal T2 imaging; coronal MPGR (Multi-Planer Gradient Echo); Axial T2* Contrast Enhanced Perfusion (0.2 ml/Kg Magnevist contrast by power injection). All animals were euthanized 10 days post injury and perfused with 4% para-formaldehyde through the ascending aorta 8 days post-injury. The brain was removed and postfixed in the same fixative solution overnight at 4ºC. After a PBS rinse, the brains were placed in 30% sucrose at 4°C before they were snap-frozen in O.C.T and stored at -80ºC. Coronal sections of the injured area were cut into 20 μm thick sections using a cryostat, mounted, and kept frozen until use. Sections were collected every 0.5 mm through all injured area over a total distance of 2 cm. Histological staining and analysis is being completed.

Figure 1. Representative histologic cross sections of brains for control (noon-treated animals), immediate treatment, a 3 hour delay between injury creation and vacuum application, or a 6 hour delay between injury creation and vacuum application. The large area of hemorrhage and necrosis and be seen in the non-treated brain. Much smaller areas are present in the immediate (0 hour delay) and the 3 hour delay animal. A small area of hemorrhage and damage is present in the 6 hour delay brain.



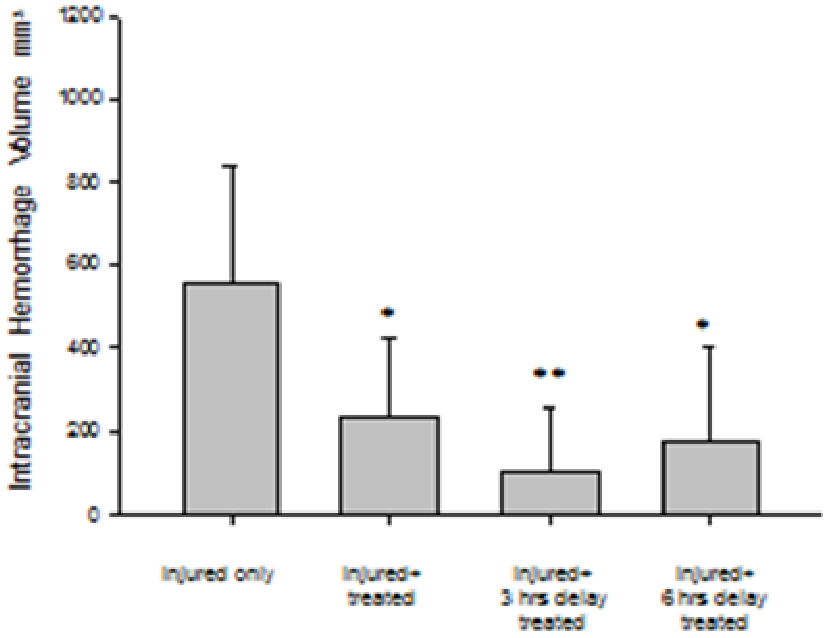
Representing images of H&E Staining in traumatic injured pig brains. Pigs were treated under negative pressure 100 mmHg for 5 days with different application start times.
A: Injured only; B: Injured brain treated without delay; C: 3 hrs delay treated; D: 6 hrs delay Treated.

Figure 2. MRI T2 analysis of hyperintense areas of the image of the injured areas showed areas of increased water content (swelling), with largest volume in the non-treated animals. The volume of the area of injury for animals treated immediately was not significantly ($p<0.05$) different that the volume for animals treated after a 3 hour delay before vacuum initiation. The volume of the injured area for animals treated following a 6 hour delay between injury and vacuum application was not significantly smaller than the non-treated animals. The volume for the 6 hour delay animals was also not significantly larger than the areas of injury for the 0 hour or 3 hour delay animals.



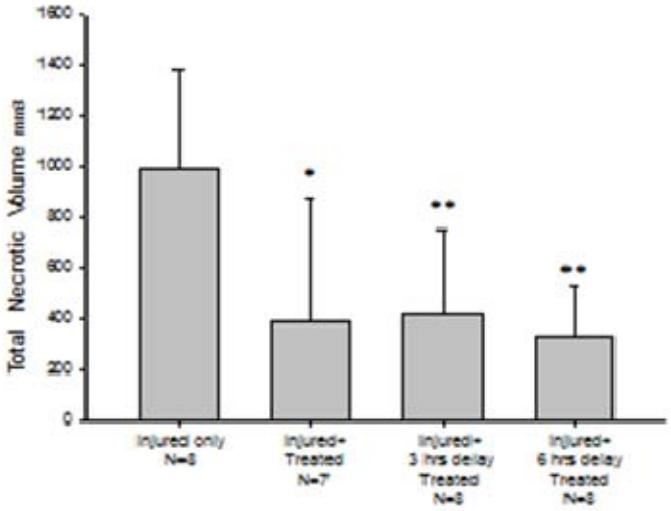
Total brain tissue injured volume measured in T2 MRI images in traumatic brain injured pigs. Pigs were treated under negative pressure 100 mmHg for 5 days with different application start time.
* Indicates P<0.05

Figure 3. Gradient echo MR imaging was used to measure the volume of the hemorrhage in the animals. All treated animals had a significantly smaller volume of hemorrhage than the non-treated animals. The volume of the hemorrhage for the 0 hour delay and the 6 hour delay were similar, with the same degree of significance ($p<0.05$). The volume for the 3 hour delay was very significantly smaller ($p<0.01$) than the non-treated volume. The volumes of hemorrhage for the treated animals were not significantly different from each other.



Total intracranial hemorrhage volume measured in gradient echo MRI images in traumatic brain injured pigs.
Pigs were treated under negative pressure 100 mmHg for 5 days with different application start times.
* indicates P<0.05; ** indicates P<0.01

Figure 4. Histologic cross sections were imaged and the volume of the area of necrosis was determined. The volume of the area of necrosis for all treated animals was significantly ($p<0.05$) smaller than the area of necrosis for non-treated animals. The volume of area of necrosis for animals in the 3 hour and 6 hour delay groups was very significantly ($p<0.01$) than the area for the non-treated animals. The area of necrosis between the 0 hour delay and the 3 or 6 hour delay was not significantly different.



Total necrotic volume in series of histological sections of traumatic brain injured pigs. Pigs were treated under negative pressure 100 mmHg for 5 days with different application start times.
* indicates P<0.05; ** indicates P<0.01

Table 1. Quantitative Table with data and maple sizes for the data presented graphically above. The sample size for histologic determination of the necrotic brain volume for the 0 hour delay group was 7 animals. All other groups contained 8 animals.

Damaged Volumes (mm3)	Injured Only Mean±SD	No Delay Treated Mean±SD	3-hrs Delay Treated Mean±SD	6-hrs Delay Treated Mean±SD
Injured Brain Tissue volume (T2 MR)	7900.72±2248.78 N=8	5621.47±1435.46 N=8	5004.30±2202.92 N=8	6919.37±2038.23 N=8
Intracranial Hemorrhage Volume (GE MRI)	553.18±283.40 N=8	232.69±194.47 N=8	102.94±154.47 N=8	175.59±229.87 N=8
Brain Necrotic Volume (H&E Staining)	985.81±395.64 N=8	388.21±486.26 N=7	414.91±341.93 N=8	338.21±195.69 N=8

Mean damaged brain volume measurements in MR images and H&E staining in traumatic brain injured pigs. Pigs were treated under negative pressure 100 mmHg for 5 days with different application start times.

Trends apparent from the MRI data show that a 3 hour delay provides for a better result than animals treated after a 6 hour delay in vacuum application. The area of injury as determined from the T2 imaging analysis exhibited a significantly smaller are of injury in animals treated after a 3 hour delay compared to animals treated after a 6 hour delay. Animals treated after a 6 hour delay still exhibited a smaller area of injury and a smaller hemorrhage volume compared to non-treated animals.

Histologic analysis of the area of necrosis shows that animals treated after the 6 hour delay had a non-statistically significant smaller area of necrosis than animals treated after a 3 hour delay. All treated animals had a statistically smaller area of necrosis than non-treated animals.

The data shows that animals treated with 100 mm Hg vacuum applied continuously for 5 days and after a 3 hour delay between injury and vacuum application exhibited as good of a result as those animals treated immediately post injury. Animals treated after a 6 hour delay between injury and vacuum application still exhibited a favorable result, although it may not have been as efficacious as the 3 hour delay group.

Key Research Accomplishments

- Determination that application of 100 mm Hg sub-atmospheric pressure for 5 days to the site of cortical injury following a 3 or 6 hour delay between injury and application of the vacuum was still efficacious. Animals in the 3 hour delay group showed a trend for a more favorable result compared to animals in the 6 hour delay group.

Reportable Outcomes

Presentation:

Zheng Z, Argenta L, Morykwas M. Mechanical Tissue Resuscitation treatment reduces injured brain tissue swellings and intracerebral hemorrhages in a pig traumatic brain injury model (delay treatment). (Abstract) Congress of Neurological Surgeons 2012 Annual Meeting, October 4 - 10, 2012

Accepted for publication (not yet published):

Johnston ME, Zheng Z, Maldjian JA, Whitlow CT, Morykwas MJ, Jung Y: Cerebral Blood Flow Quantification in Swine using Pseudo-Continuous Areterial Spin Labeling, Journal of Magnetic Resonance Imaging.

Conclusion

This portion of the study demonstrates that the use of mechanical tissue resuscitation (MTR) treatment of a continuous vacuum of 100 mm Hg for 5 days was as efficacious after a 3 or 6 hour delay between injury creation as compared to animals treated immediately after injury creation (0 hour delay).

The expectation of rapid translation of the technique to humans is still anticipated with interest from industry for commercialization of the product and technique, although no formal licensing discussions have begun.

References

N.A.

Appendices

N.A.